# APPENDIX F


# Scientific Report

April 17th 2002

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April 17th 2002

Dear colleague,

I would like to take advantage of this opportunity to present to you the updated Scientific Report of Neptune Technologies & Bioressources Inc. In the next few pages, you will find a brief description of the primary Neptune extraction process designated as "Neptune OceanExtract™" followed by an introduction to the three initial products, "Neptune Krill Oil™", "Neptune Aquatein™" and "Neptune LyO-Krill™". You will also find a summary of the medical research performed with Neptune Krill Oil™. If you require a more specific profile of our products, it will be a pleasure to send it to you within a short delay.

Should you have any questions or comments, please feel free to contact me at Neptune Technologies & Bioressources Inc. main office or via e-mail at tinas@neptunebiotech.com.

Sincerely,

Tina Sampalis MD, PhD.
Vice President, Research
Neptune Technologies & Bioressources Inc.
500 Saint-Martin Blvd. West, suite 550,
Laval (Québec) H7M 3Y2
Tel. (450) 972-6291 1-888-664-9166
Fax (450) 972-6351

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## NEPTUNE OCEANEXTRACT™

During 1998–1999, Neptune Technologies & Bioressources Inc. developed, in experimental phase at University of Sherbrooke, an extraction process **(Neptune OceanExtract™)** of natural health products with high nutritional value from marine biomasses. Krill was the first biomass used with this new process followed by seal, whole fish, fish residue and others. Subsequently, the procedure on krill was brought to a pilot industrial level in collaboration with the CRIQ. Neptune recently announced the initiation of the first phase of its industrialization plan and production is scheduled to begin in June 2002 under GMP and GLP compliance.

## PROCEDURE

**Neptune OceanExtract™** is a cold extraction process, allowing the extraction of:

a) **Neptune Krill Oil™** : an oil rich in Omega-3, phospholipids and potent antioxidants,
b) **Neptune Aquatein™** : a protein concentrate containing all essential amino acids.

## ADVANTAGES OF "NEPTUNE OCEANEXTRACT™"

- This cold extraction process allows the preservation of the biological activity of all krill components;
- Neptune OceanExtract™ process is effective in the destruction of bacteria, offering a secure product for human consumption;
- Lipid alterations are minimal;
- Achieves complete utilization of the biomass allowing a high yield and minimal waste;
- Offers significant stability of the final products without the use of additive antioxidants or preservatives;
- Produces non-oxidized (peroxide value = 0) high quality products, rich in essential nutrients, highly stable, bacterial free and thus, safe for human consumption with noteworthy health benefits.

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# NEPTUNE PRODUCTS

## NEPTUNE KRILL OIL™

**Neptune Krill Oil™**, a marine oil offering a unique and natural mixture of essential nutrients. It is characterized by its high content of polyunsaturated fatty acids such as Omega-3 and 9, potent antioxidants such as astaxanthin, canthaxanthin, vitamin A, vitamin E as well as a novel flavonoid and unique phospholipids such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol and sphingomyelin while demonstrating a remarkable and naturally acquired stability without the addition of any preservatives or antioxidants.

## NEPTUNE AQUATEIN™

**Neptune Aquatein™** , the dry fraction (or residue) remaining after the extraction of Neptune Krill Oil™. This residue's main characteristic features are its high protein content, 20 common amino acids including all essential amino acids, active and stable enzymatic activity, traces of residual pigments, traces of polypeptides (more or less short chains of amino acids resulting from the protein's self-digestion), chitin and growth agent.

## NEPTUNE LYO-KRILL™

**Neptune LyO-Krill™**, a marine biomass product with preserved bioactive ingredients in the form of natural antioxidants such as: vitamin A, vitamin E, vitamin B1, niacin as well as axtaxanthin and canthaxanthin. Combined with P.U.F.A's and naturally enriched with calcium, copper and phospholipids, LyO-Krill™ represents an exceptional multi-functional dietary supplement, rich in essential amino acids, Branch Chained Amino Acids, digestive enzymes and peptides.

## NEPTUNE KRILL ENZYMES™

**Neptune Krill Enzymes™**, a new marine enzyme supplement with 80% protein that contains 20% amino acids including 10 essential and 17% of BCAA (Branch Chained Amino Acids: Leucine, Isoleucine, Valine). Natural powerful digestive enzymes like proteases, phosphatases and phosphohydrolases combined with peptides that have potent biological activity offer a natural health product that can facilitate digestion and healing in multiple burn and trauma patients.

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## NEPTUNE KRILL OIL™

### 1. OMEGA-3 FATTY ACIDS

#### a) Omega-3 fatty acids

Neptune Krill Oil™ contains a high quantity of Omega-3 fatty acids (EPA / DHA). The distribution of these Omega-3 fatty acids is what distinguishes Neptune Krill Oil™ as unique among the family of Omega-3 oils.

Scientific evidence proves that:

- DHA and EPA play a key role and may be beneficially supplemented for depression, schizophrenia, diabetes, cancer, rheumatoid arthritis and atherosclerosis (1, 2, 3);
- Omega-3 fatty acids (EPA & DHA) have a beneficial effect on dysmenorrhoea in adolescents (4);
- The effects of Omega-3 fatty acids supplementation in obese people and patients with arterial disease is verified in relation to genetic variation.

#### b) Omega-3 : Omega-6 ratio

Neptune Krill Oil™ offers an ideal ratio of Omega-3 : Omega-6, significantly favouring the Omega-3 which is deficient in our everyday diet.

- This ratio inhibits the effects of excess Omega-6 fatty acids, especially arachidonic acid, which is associated with chronic disease (5);
- Neptune Krill Oil™ contains 0.00 – 0.41 grams of arachidonic acid per 100 grams of oil.

#### c) Omega-9 (oleic acid)

Scientific evidence suggests that:

- Oleic acid slows down the gastrointestinal transit for patients with short bowel disease (38). .

### 2. Potent antioxidants

#### a) Vitamin A – all-trans retinol

Scientific evidence suggests that:

- Vitamin A can reverse cell and tissue changes during neoplastic transformation indicating a potential role in cancer prevention (6);
- Retinol, beta-carotene, along with other dietary carotenoids, function as antioxidants that can prevent cellular damage at all stages from aging to carcinogenesis by decreasing the levels of the free-radicals that cause DNA damage (7).

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**b) Vitamin E – alpha-tocopherol**

Scientific evidence suggests that:

- Vitamin E may help prevent or delay coronary heart disease by inhibiting LDL- cholesterol oxidation and thrombus formation (8);
- Antioxidants such as vitamin E help protect against the damaging effects of free radicals, which may contribute to the development of chronic disease such as cancer (9).

**c) Astaxanthin - esterified 3R-3R/Canthaxanthin**

Astaxanthin has been proven to be:

- Twice as effective as beta-carotene (and about 80 times more effective than vitamin E) in quenching singlet oxygen in chemical solution (13);
- 50% more effective than beta-carotene and zeaxanthin, in preventing fatty acid peroxidation in chemical solution (14);
- In a membrane model, astaxanthin was found to be more effective at scavenging peroxyl radicals than was beta-carotene (15).

**d) Flavonoids**

Scientific evidence has shown that:

- Flavonoid antioxidant activity is accepted as a scientific fact (16-19);
- Epidemiological, clinical, and laboratory research on flavonoids, demonstrate the use of flavonoids in the prevention and/or treatment of cardiovascular disease, cancer, inflammatory conditions, asthma, periodontal disease, liver disease, cataracts and macular degeneration (17,18);
- Until today, there has never been a flavonoid extracted from anything other than plant, vegetable, fruit or algae.

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3. **Phospholipids (PL)**

| | **Phospholipids** | **46 g/100g** |
|---|---|---|
| **(PC)** | Phosphatidylcholine | 24.0 |
| **(PS)** | Phosphatidylserine | TBA |
| **(PI)** | Phosphatidylinositol | 4.8 |
| **(SM)** | Sphingomyelin | TBA |
| **(PE)** | Phosphatidylethanolamine | 8.9 |

Phospholipids available in the market are derived from plant, egg yolk or cows (brain or liver);
Scientific evidence suggests that:

- Soy-based PC contain linoleic acid and alpha-linolenic acid as fatty acids;
- While plant-based PC have some beneficial effects on the brain, their fatty acids profiles are not ideal since they are different than those in the human brain;(20)

According to Dr. Michael Schmidt, krill-based phospholipids can be regarded as the preferred phospholipids for supporting peak brain performance due to their high content of polyunsaturated Omega-3 (EPA/DHA) fatty acids (20).

4. **High natural stability**

a. **Peroxide value 0.00**
This is the classic test for measuring oxidation in fresh oils. A peroxide value over 2 is an indicator that the product has a high rancidity potential and could fail on the shelf.

b. **Oil stability index Peroxide value <0.1 for more than 50 hours at 97.8°C**
All oils and fats have a resistance to oxidation, which depends on the degree of saturation, natural or added antioxidants or prior abuse. Oxidation is slow until this resistance is overcome, at which point oxidation accelerates and becomes very rapid. The length of time before this rapid acceleration of oxidation is the measure of the resistance to oxidation and is commonly referred to as the "induction period" or "Oxidative Stability Index".

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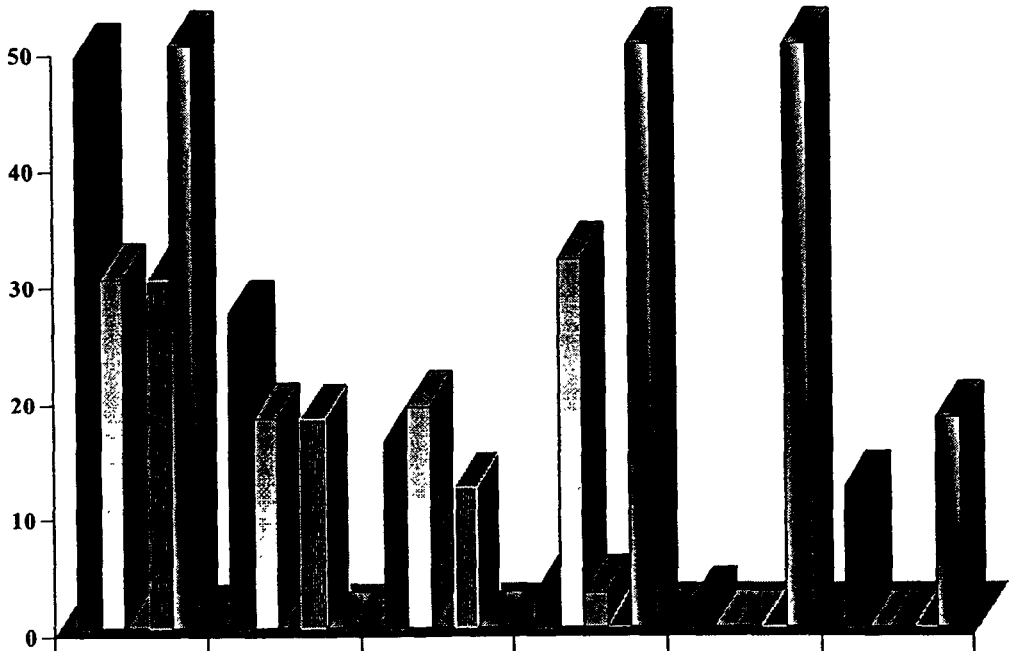
Preliminary results
Pilot tests
2002

## Competition

# Neptune Krill Oil™ vs. Other Oils

[g/100g]




| Oils | omega-3 | EPA | DHA | omega-6 | linoleic | oleic omega-9 |
|---|---|---|---|---|---|---|
| ■ Krill | 49 | 27 | 16 | 3 | 2 | 12 |
| □ Fish | 30 | 18 | 19 | 31,6 | 0 | 0 |
| ▨ Macademia | 0 | 0 | 0 | 3 | 0 | 0 |
| ■ Cod Liver | 30 | 18 | 12 | 0 | 0 | 0 |
| ■ Flax | 56 | 0 | 0 | 70 | 70 | 18 |

Date of analysis: Feb. 08,2002

Fig. 1: Comparison of Neptune Krill Oil™ Omega fatty acids with other natural oils in the market.

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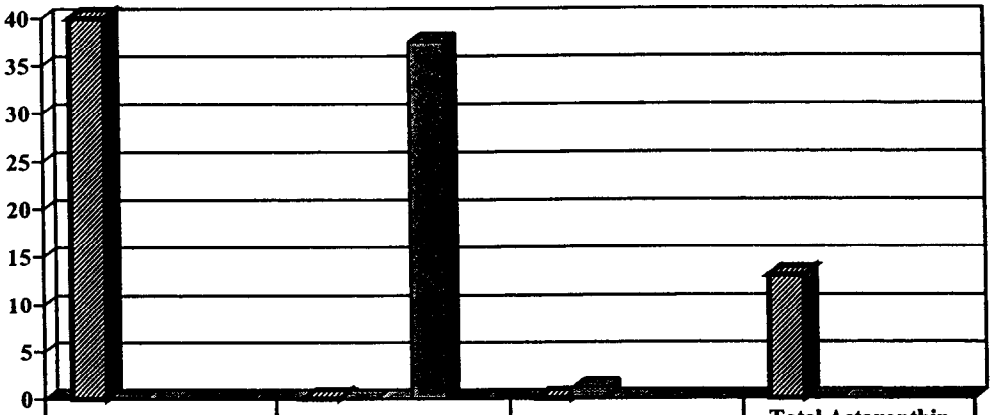
Preliminary results
Pilot tests
2002

## Neptune Krill Oil™ vs. Other Oils
[g/100g]




| | PL | Vit. A | Vit. E | Total Astaxanthin Deriv. |
|---|---|---|---|---|
| Krill | 46 | 0,024 | 0,074 | 13 |
| Fish | 0 | 0 | 1,1 | 0 |
| Macadenia | 0 | 0 | 0 | 0 |
| Cod Liver | 0 | 37,5 | 0 | 0 |
| Flax | 0 | 0 | 0 | 0 |

Date of analysis: Feb. 08, 2002

Fig. 2: Comparison of Neptune Krill Oil™ phospholipids and antioxidants with other natural oils in the market.

The above histograms (Fig. 1 and 2):

- Demonstrate the advantages of Neptune Krill Oil™ versus other popular natural oils in the market;
- The alternative products and main competitors are fish oil, cod liver oil and flaxseed oil.
    - Fish oil contains a high quantity of Omega-3 and EPA / DHA in an excellent proportion but only a 1 : 1 Omega-3 : Omega-6 ratio. Furthermore, it does not contain antioxidants;
    - Cod liver oil has an excellent Omega-3 EPA / DHA content and very high vitamin A but does not contain phospholipids and offers none of the more potent antioxidants like astaxanthin and canthaxanthin;
    - Flaxseed oil contains a high quantity of alpha-linolenic acid, an Omega-3 fatty acid, but no EPA or DHA. In addition, the Omega-3:Omega-6 ratio is unfavourably reversed, in support of Omega-6.

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## NEPTUNE AQUATEIN™

**Neptune Aquatein™** , the dry fraction (or residue) remaining after the extraction of Neptune Krill Oil™. This residue's main characteristic feature is its high protein content of Antarctic Krill *Euphausia superba.* It offers the following genuine and potential attributes : high protein content, 20 common amino acids, all essential amino acids, active enzymatic activity, traces of residual pigments, traces of polypeptides (more or less short chains of amino acids resulting from the proteins self-digestion), chitin and traces of a growth agent.

### PROTEINS

- Krill proteins are made of 20 common amino acids the contents of which are typically displayed (or described) in the manufacturers' specification sheets;
- The pure proteins value in Neptune Aquatein™ is 83%.

### ENZYMES

- Scientific research has shown that Aquatein™ enzymes:
    - Are: lipases, phospholipases, alkaline phosphatase, acid phosphatase, esterase, trypsine, phosphohydrolase, â-glucoronidase, á-glucosidase, proteases, hyalurinases, and nucleases;
    - Have uncommonly high enzymatic activity;
    - React in low temperatures.

### CHITIN

- The chitin and chitosan content of krill is, on a dry basis, between 2.4% to 2.7% and 2.8 respectively;
- Chitin is the source of a high-value added biopolymer chitosan with applications in the biomedical and pharmaceutical industries.

### PEPTIDES

- Peptides correspond to pre-digested proteins, which, if taken raw, facilitate the digestion and assimilation;
- Peptides correspond to sections of proteins issued from the action of proteolytic enzymes contained in krill;
- These sections are chains of amino acids more or less short;
- The presence of noticeable contents of polypeptides adds nutrient value to the Neptune protein concentrate (Neptune Aquatein™).

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## NEPTUNE LYO-KRILL™

**Neptune LyO-Krill™**, a rich and well balanced source of essential nutrients: polyunsaturated fatty acids (P.U.F.A.), amino acids, phospholipids, vitamins and minerals. The superior quality of our product is attributed to the presence of intact absorbable, pre-digested proteins and biologically active polyunsaturated fatty acids, especially EPA and DHA and active enzymes. This condition ensures optimal biological action.

### AMINO ACIDS, ENZYMES, PEPTIDES

- 20 amino acids, including all the essential amino acids, with 16% of BCAA (Branch Chained Amino Acids);
- The enzymes are: proteases, alkaline and acid phosphatases which are powerful, digestive and hydrolytic;
- Special peptides are present and have potent biological activity.

### PHOSPHOLIPIDS

- Five phospholipids;
- May boost memory and improve concentration, learning, mood and overall well-being;
- May help to decrease the effects of neurodegenerative diseases (20).

### OMEGA –3

- Omega-3 fatty acids are proven to have a significant beneficial effect on inflammatory and cardiovascular disease (1-4).

### ASTAXANTHIN AND CANTHAXANTHIN

- Astaxanthin is recognized as one of the most potent natural antioxidant;
- Beneficial for cardiovascular diseases, primary cancer prevention, anti-aging, neurodegenerative diseases and ophthalmic disorders (13-15).

### VITAMINS

- Vitamin A (all-trans retinol) , an essential fat-soluble vitamin with proven photoprotective and anti-aging potential (6,7).

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## RESEARCH

### CARDIOVASCULAR DISEASE

- A recent study performed by Andrioli et al.(21) indicated that the systemic administration of fish oil rich in Omega-3 fatty acids inhibits platelet adhesion and thus plaque formation with an important determinant being the Omega-6 / Omega-3 ratio (17);
- Meydani showed that Omega-3 polyunsaturated fatty acids in fish oil have protective effects on cardiovascular disease by reducing the vascular endothelial inflammation in atherosclerosis;
- Postoperative daily administration of Omega-3 fatty acids in heart transplant recipients is effective as hypertension prophylaxis as proven by Guardia et. al.(18,21);
- Furthermore, astaxanthin has been shown in both *in vitro* experiments and in human subjects to be effective for the prevention of the oxidation of low density lipoprotein. Cos et al. (19).

The increased content of Omega-3 and antioxidants in Neptune Krill Oil™ allows us to presume beneficial effects for the maintenance of a healthy cardiovascular system.

Neptune is presently conducting a series of prospective randomized double blind trials in order to investigate the health benefits of Neptune Krill Oil™ on cardiovascular disease.

### NEOPLASTIC DISEASE

- Retinols have been proven to have prophylactic effects against UV radiation induced skin cancer (6);
- Astaxanthin has been proven to be twice as effective as beta-carotene (and about 80 times more effective than vitamin E) in quenching singlet oxygen in chemical solution (13);
- 50% more effective than beta-carotene and zeaxanthin, in preventing fatty acids peroxidation in chemical solution (14);
- In a membrane model, astaxanthin was found to be more effective at scavenging peroxyl radicals than was beta-carotene (15,23).

The high content of all-trans retinols and astaxanthin in Neptune Krill Oil™ justifies further investigations of possible anticarcinogenic properties of our product preparation.

Neptune is conducting a study to evaluate the photoprotective potential of Neptune Krill Oil™ against UVB-induced skin cancer.

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## RHEUMATOID ARTHRITIS

- Piet Geusens et al. showed that eicosanoids have an inhibitory effect on the formation of 2-series prostanoids and 4-series leukotrienes resulting in an improvement in the severity of symptoms and a decrease in the amount of corticosteroids and NSAIDS consumed (24);

- These observations are confirmed by Kremer et al., who demonstrated that Omega-3 fatty acids significantly inhibits the production of IL-1 (3).

Neptune is conducting a research study in order to evaluate the potential effects of Neptune Krill Oil™ supplementation on the clinical course of rheumatoid arthritis.

## FACIAL WRINKLE REDUCTION

- The main property of retinol is the significant increase in cell differentiation. Based on their regenerative properties, retinols have been proven to be effective in the reduction of wrinkles when used topically (25, 26);

- Astaxanthin preparations have been shown to be efficient for the prevention of light aging of skin (23).

Neptune Krill Oil™ is a natural source of retinol and astaxanthin combined which could potentially increase their efficiency.

Neptune is conducting a research project in order to measure the effects of Neptune Krill Oil™ on aging and facial wrinkles.

## TRANSDERMAL TRANSPORT

- Santoyo and Ygartua (28) demonstrated that percutaneous absorption can be enhanced with the topical use of fatty acids and phospholipids (24,25).

The high fatty acid and phospholipid composition and the significant all-trans retinol content with vitamin E in Neptune Krill Oil™ may facilitate the transdermal transportation of creams, ointments, gels or lotions.
Neptune is testing these properties on experimental models (nude mice) with skin analogous to that of human skin. The objectives of this study will be to evaluate the efficacy of Neptune Krill Oil™ as a substrate for topical treatments and to verify the speed of transdermal absorption of Neptune Krill Oil™ alone or as a substrate to other products in the cosmeceutical and/or biopharmaceutical industries.

**Neptune Technologies & Bioressources Inc., recognizing the trend of modern medicine is investing in Research & Development of natural, efficient and pure products for the nutraceutical, biopharmaceutical and cosmeceutical industries.**

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